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IN-DEPTH, COMPREHENSIVE MAPPING OF THE HUMAN SEMINAL PLASMA PROTEOME BY A NOVEL, ITERATIVE LC-MS/MS/DATABASE SEARCH WORKFLOW

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Apart from its obvious role in transporting male gametes, the seminal plasma provides a protective environment for ejaculated spermatozoa and improves their fertilizing potential. This fluid is also a potential source of biomarkers for male reproductive disorders. Here we describe a method for enhanced protein identification in complex samples. A combination of iterative LC-MS/MS, exclusion list generation (including masses and retention time) and iterative database searching was used to analyze in depth the seminal plasma proteome.

Non-liquefied seminal plasma (500mg proteins) from a healthy donor was loaded onto two sequential hexapeptide ligand libraries (1ml each; ProteoMiner™ –primary amino terminal peptides– and a carboxylated form, Bio-Rad Laboratories). Proteins bound on both libraries were desorbed through 4 different elution buffers, generating 8 complementary and treated sub-proteomes. Each fraction was then trypsin-digested and analysed by nano-LC-MS/MS with an LTQ Orbitrap XL™ mass spectrometer (Thermo Fisher Scientific). Peptides were preconcentrated with a peptide Captrap cartridge (MICHROM Bioresources, Inc.) and separated onto a 15cm x 100µm capillary column. Detected peptides were selected for CID fragmentation using data dependent criteria and MS/MS spectra were searched against a SwissProt human database (April 2008) for peptide characterisation and protein identification. A list of peptide masses resulting from database searching was generated with Proteome Discoverer™ for each sample and these identified peptide masses used to generate an exclusion list, including retention times, for subsequent LC-MS analysis of the same sample. A second database search was performed. Identified peptide masses from the first and second LC-MS experiments were combined to generate a second, longer exclusion list to be used for a third round of mass spectrometry analysis and subsequent database searching and peptide and protein identification.

This procedure resulted in the identification of many additional peptides in the seminal plasma sample. More than 80% of the peptides identified in run 2 and 3 were new and could be used to confirm “one hit wonder” protein hits, increase protein coverage or identify new proteins. A total of 864 proteins were identified at a 5% FDR demonstrating the benefit of this strategy for increasing the dynamic range of identified proteins toward a deep proteomic characterization of seminal plasma.